

## Comparative Analysis of Different Approaches to Measure Treatment Response in Acute Myeloid Leukemia

Hiroto Inaba, Elaine Coustan-Smith, Xueyuan Cao, Stanley B. Pounds, Sheila A. Shurtleff, Kathleen Y. Wang, Susana C. Raimondi, Mihaela Onciu, Jeffrey Jacobsen, Raul C. Ribeiro, Gary V. Dahl, W. Paul Bowman, Jeffrey W. Taub, Barbara Degar, Wing Leung, James R. Downing, Ching-Hon Pui, Jeffrey E. Rubnitz, and Dario Campana

Hiroto Inaba, Xueyuan Cao, Stanley B. Pounds, Sheila A. Shurtleff, Kathleen Y. Wang, Susana C. Raimondi, Mihaela Onciu, Jeffrey Jacobsen, Raul C. Ribeiro, Wing Leung, James R. Downing, Ching-Hon Pui, and Jeffrey E. Rubnitz, St Jude Children's Research Hospital and University of Tennessee College of Medicine, Memphis, TN; Elaine Coustan-Smith and Dario Campana, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; Gary V. Dahl, Lucile Packard Children's Hospital and Stanford Cancer Center, Palo Alto, CA; W. Paul Bowman, Cook Children's Medical Center, Fort Worth, TX; Jeffrey W. Taub, Children's Hospital of Michigan, Detroit, MI; and Barbara Degar, Dana-Farber Cancer Institute, Boston, MA.

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Corresponding author: Hiroto Inaba, MD, PhD, Department of Oncology, MS 260, St Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105-3678; e-mail: hiroto.inaba@stjude.org.

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### ABSTRACT

#### Purpose

In acute myeloid leukemia (AML), initial treatment response by morphologic analysis of bone marrow predicts long-term outcome. Response can now be assessed by minimal residual disease (MRD) monitoring with flow cytometry or polymerase chain reaction (PCR). We determined the relation among the results of these approaches and their prognostic value.

#### Patients and Methods

In the multicenter AML02 study, follow-up bone marrow samples from 203 children and adolescents with newly diagnosed AML were examined by flow cytometry ( $n = 1,514$ ), morphology ( $n = 1,382$ ), and PCR amplification of fusion transcripts ( $n = 508$ ). Results were correlated with treatment outcome.

#### Results

Among 1,215 samples with less than 5% leukemic myeloblasts by morphology, 100 (8.2%) were MRD positive ( $\geq 0.1\%$ ) by flow cytometry, whereas 96 (57.5%) of the 167 samples with  $\geq 5\%$  blasts were MRD negative. Virtually all (308 of 311; 99.0%) MRD-negative samples by PCR were also MRD negative by flow cytometry. However, only 19 (9.6%) of the 197 PCR-positive samples were flow cytometry positive, with analyses of *AML1-ETO* and *CBF $\beta$ -MYH11* accounting for most discrepancies, whereas eight of 13 *MLL*-positive samples had detectable MRD by flow cytometry. MRD by flow cytometry after induction 1 or 2 predicted lower event-free survival and higher relapse rate ( $P < .001$ ) and was an independent prognostic factor in a multivariable analysis; prediction was not improved by morphologic information or molecular findings.

#### Conclusion

In childhood AML, morphologic assessment of treatment response has limited value if MRD is measured by flow cytometry. *MLL* fusion transcripts can provide prognostic information in some patients, whereas monitoring of *AML1-ETO* and *CBF $\beta$ -MYH11* transcripts is largely uninformative.

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### INTRODUCTION

In patients with acute leukemia, assessment of early treatment response predicts relapse hazard and is used for risk-directed therapy.<sup>1-6</sup> Residual leukemic cells are traditionally identified morphologically, but this task is difficult owing to their similarity to normal hematopoietic cells. Methods to track leukemic cells based on flow cytometry or polymerase chain reaction (PCR) have the potential for superior sensitivity and accuracy; they allow the recognition of leukemic cells present at levels well below those detectable by morphology (ie, minimal residual disease [MRD]).<sup>1-6</sup> In acute myeloid leukemia (AML), flow cytometric detection of MRD predicts outcome.<sup>7-13</sup> Likewise, increases in levels of onco-

genic fusion transcripts measured by PCR are reportedly associated with a higher probability of relapse in patients with specific genetic subtypes of AML.<sup>14-23</sup> Our recent multi-institutional study of children and adolescents with AML (AML02) used flow cytometric assessments of MRD to guide therapy and resulted in high overall cure rates, suggesting that this approach can contribute to improving clinical management.<sup>24</sup>

The clinical utility of morphologic assessment of treatment response in the MRD era is unclear. Moreover, virtually all MRD studies in AML reported to date have relied on one single technique; hence, the relation between MRD results obtained by flow cytometry and those obtained by PCR is unknown. A better understanding of the relative

prognostic strength of each approach is critical to the interpretation of the prognostic significance of early treatment response and designing of risk assignment strategies. We report here the results of flow cytometric monitoring of MRD in 1,514 bone marrow samples obtained from 203 patients enrolled onto the AML02 study during and after completion of therapy. We compared the results with those of morphology and PCR and correlated the results with treatment outcome.

## PATIENTS AND METHODS

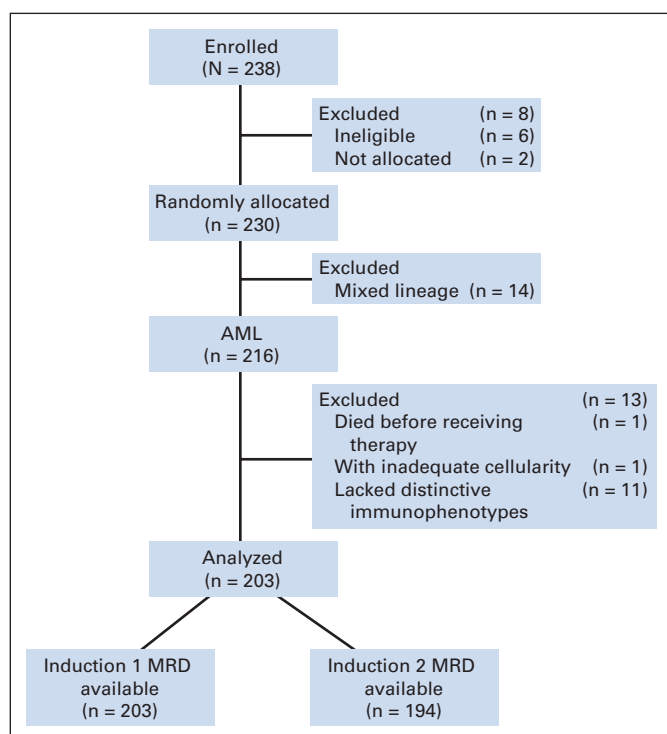
### Patients and Treatment Protocol

From October 2002 to June 2008, 216 patients with AML were enrolled onto the AML02 study at seven centers.<sup>24</sup> Of these patients, one died before receiving therapy. Among the remaining 215, 11 (5.1%) lacked distinctive immunophenotypes at diagnosis for an MRD assay with at least 0.1% sensitivity, and one had follow-up samples with inadequate cellularity. Thus, the data reported are from 203 patients (Fig 1). The study was approved by the participating institutional review boards; informed consent was obtained from the patients or their parents or legal guardians, and assent was given by the patients, as appropriate.

Cytogenetic analysis and reverse transcriptase-PCR to detect the presence of fusion transcripts were performed at diagnosis.<sup>8,25</sup> The AML02 treatment protocol was previously reported<sup>24</sup> and is summarized in the Data Supplement.

### Morphologic Assessment of Treatment Response and Studies of MRD

The first bone marrow evaluation was performed on day 22 of induction 1. If more than 7 days passed between day 22 and blood count recovery (absolute neutrophils  $\geq 300/\mu\text{L}$ ; platelets  $\geq 30,000/\mu\text{L}$ ), a second evaluation was performed to determine response to induction 1. Additional evaluations were performed after each phase of treatment.



**Fig 1.** Outline of patient enrollment onto the study. AML, acute myeloid leukemia; MRD, minimal residual disease.

Bone marrow aspirate smears were examined for morphologic indication of disease. For flow cytometric detection of MRD, bone marrow aspirates were processed as previously described (Data Supplement).<sup>24</sup> Patients with a fusion transcript at diagnosis were monitored by PCR (Data Supplement).

### Statistical Analysis

Event-free survival (EFS) was defined as the time elapsed from study enrollment to induction failure, withdrawal, relapse, secondary malignancy, or death. The association between presenting features and MRD was assessed using Fisher's exact test with Monte Carlo simulation. The Kaplan-Meier method was used to estimate the probability of EFS, and SEs were determined by the method of Peto et al.<sup>26</sup> Survival comparisons were performed by Mantel-Haenszel log-rank test.<sup>27</sup> A multivariable analysis of EFS was performed using Cox proportional hazards regression, stratified by treatment arm.

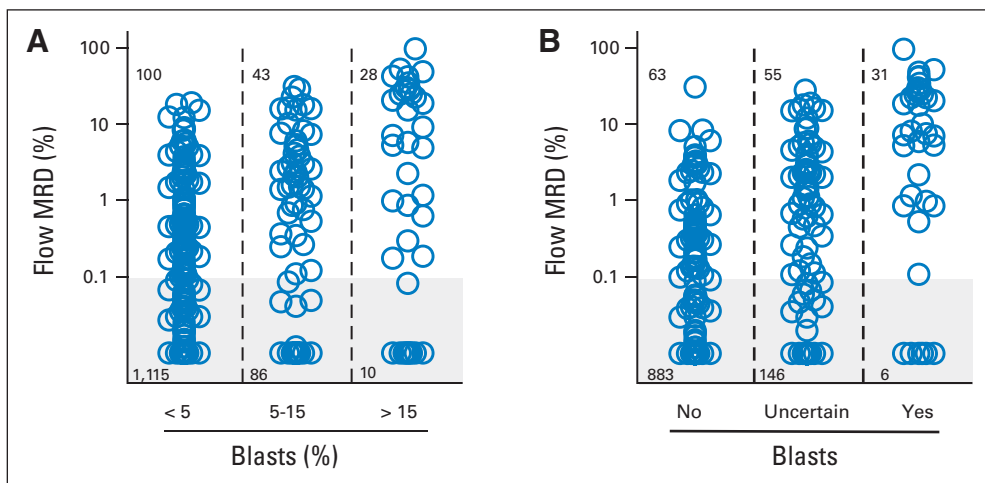
## RESULTS

### Relation Between MRD by Flow Cytometry, Presenting Features, and Morphologic Assessment of Residual Disease

Among the 1,514 bone marrow samples studied, 202 (13.3%) had  $\geq 0.1\%$  mononucleated cells expressing leukemia-associated immunophenotypes determined at diagnosis. MRD was more prevalent during the early phases of treatment (Data Supplement). The proportion of samples with MRD  $\geq 1\%$  was also higher after induction 1 (54 of 86 samples) than after induction 2 (23 of 56;  $P = .016$ ). After induction 1, MRD ( $\geq 0.1\%$ ) was more prevalent in samples with French-American-British M1 morphology ( $P = .001$ ), normal karyotype ( $P < .001$ ), and *FLT3*-internal tandem duplication (ITD;  $P < .001$ ) and less prevalent in patients with *inv(16)* ( $P < .001$ ); after induction 2, it was more prevalent in patients with *FLT3*-ITD ( $P < .001$ ) and less frequent in patients with *inv(16)* ( $P = .003$ ; Data Supplement).

Data on cell morphology were available for 1,382 (91.3%) of the 1,514 samples from 202 (99.5%) of the 203 patients. We plotted flow cytometric MRD data against morphologic blast cell counts (Fig 2A). Twenty-eight (73.7%) of the 38 samples with  $> 15\%$  myeloblasts were MRD positive by flow cytometry (median, 17.1%; range, 0.18% to 97.0%), and one had 0.08% of cells with an aberrant immunophenotype. MRD was positive in 43 (33.3%) of the 129 samples with 5% to 15% myeloblasts (median, 2.58%; range, 0.11% to 31%), with five additional samples containing 0.01% to 0.09% abnormal cells. Finally, 100 (8.2%) of the 1,215 samples with  $< 5\%$  myeloblasts were also MRD positive (median, 0.66%; range, 0.1%–19.0%); an additional 44 samples had cells expressing abnormal immunophenotypes at  $< 0.1\%$ , the threshold used to define MRD positivity by flow cytometry in this study (median, 0.03%; range, 0.01% to 0.09%).

We also compared hemopathologist assessments, available for 1,184 samples from 185 patients, regarding the presence of leukemic myeloblasts with flow cytometric results (Fig 2B). Of the 37 samples deemed to contain leukemic myeloblasts by morphology, 31 were MRD positive by flow cytometry (median, 17.9%; range, 0.11% to 97.0%), whereas six samples contained only immunophenotypically normal maturing myeloid cells. Of the 946 samples with no detectable leukemic myeloblasts by morphology, 63 (6.7%) were MRD positive by flow cytometry (median, 0.49%; range, 0.1% to 31%), and an additional 27 samples had immunophenotypically abnormal cells



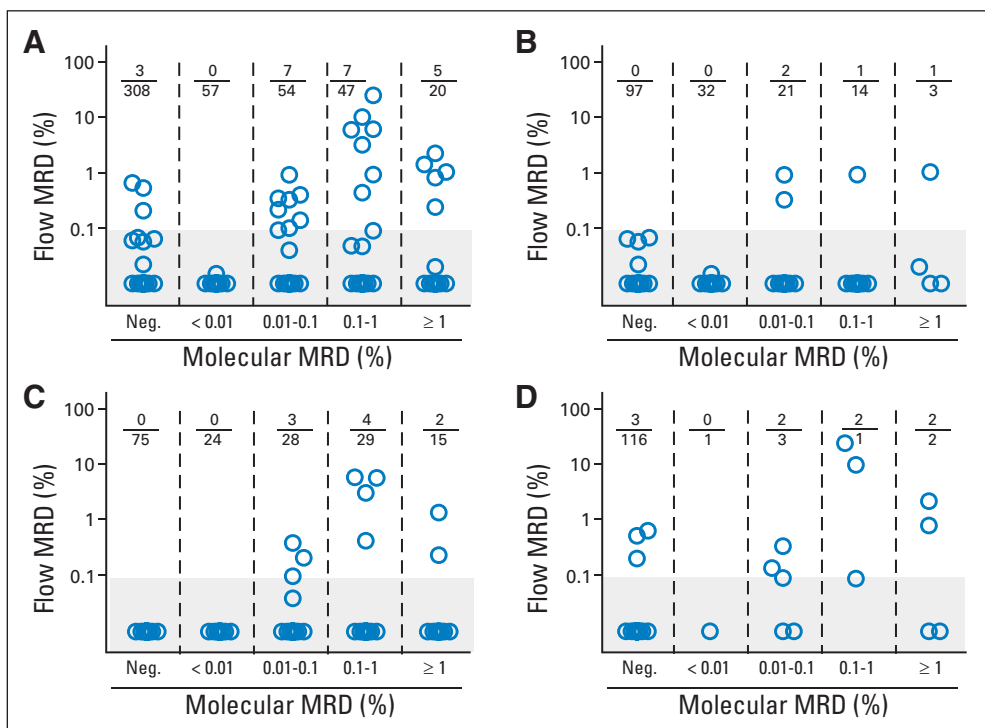
**Fig 2.** Relation between morphologic and flow cytometric detection of residual disease during and after treatment in childhood acute myeloid leukemia. (A) Percentage of bone marrow mononucleated cells expressing leukemia-associated immunophenotypes as measured by flow cytometry within groups defined by the percentage of myeloblasts counted by morphology. Shaded area encloses data points below the 0.1% threshold used to define positive minimal residual disease (MRD) in this study. (B) Flow cytometric MRD data within groups defined by hematopathologist judgment regarding the presence of leukemic myeloblasts.

at < 0.1% (median, 0.04%; range, 0.01% to 0.09%). Finally, a substantial proportion of samples (n = 201; 17.0%) were difficult to interpret morphologically, and no conclusion could be made with regard to the presence of leukemic blasts; 55 (27.4%) of these were MRD positive by flow cytometry (median, 2.1%; range, 0.1% to 28.4%); an additional 12 samples contained immunophenotypically abnormal cells at < 0.1% (median, 0.04%; range, 0.01% to 0.09%). Thus, a substantial number of samples with no morphologic evidence of disease or with uncertain morphology contained leukemic cells, whereas some samples with cells appearing morphologically as myeloblasts lacked immunophenotypic abnormalities and were most likely normal myeloid progenitors.

### MRD by PCR and Comparison With Flow Cytometry

We evaluated MRD by PCR in patients whose leukemic cells at diagnosis contained *AML1-ETO* (n = 31), *CBFB-MYH11* (n = 24), *MLL* fusion transcripts (n = 22: *MLL-AF9*, n = 12; *MLL-AF10*, n = 5; *MLL-ENL*, n = 4, *MLL-ELL*, n = 1), and *RBM15-MKL1* (n = 3; Data Supplement). Of the 1,514 samples studied by flow cytometry, 508 (33.6%) were also studied by PCR.

Of the 311 samples classified as MRD negative by PCR, 308 (99.0%) were also MRD negative by flow cytometry (Fig 3A; Data Supplement). However, in three samples from two patients (one with *MLL-AF9*; one with *MLL-AF10*), flow cytometry detected 0.21%, 0.53%, and 0.66% mononucleated cells with an aberrant



**Fig 3.** Relation between molecular and flow cytometric detection of residual disease during and after treatment in childhood acute myeloid leukemia. Percentage of bone marrow mononucleated cells expressing leukemia-associated immunophenotypes as measured by flow cytometry within groups of samples with different levels of minimal residual disease (MRD) according to analysis of (A) fusion transcripts (*AML1-ETO*, *CBFB-MYH11*, *MLL-AF9*, *MLL-AF10*, *MLL-ENL*, *MLL-ELL*, or *RBM15-MKL1*) by polymerase chain reaction. Results obtained in subgroups defined by (B) *AML1-ETO*, (C) *CBFB-MYH11*, and (D) *MLL* transcripts are also shown. Shaded areas enclose data points below the 0.1% threshold used to define positive MRD by flow cytometry in this study. Samples collected at any time point during and after treatment were included.

**Table 1.** Univariate Analysis of EFS According to Flow Cytometric, Morphologic, and Molecular Analysis of Residual Disease

Residual Disease Measurement	Induction 1				Induction 2			
	No. of Patients	5-Year EFS	± SD	P	No. of Patients	5-Year EFS	± SD	P
Flow cytometry, %				< .001				< .001
≥ 1	51	29.3	8.2		17	17.6	16.0	
0.1 to < 1	24	61.8	14.4		22	44.6	13.6	
< 0.1	128	73.4	6.1		155	70.7	5.6	
Blasts, %				.016				.058
≥ 5	73	50.2	9.5		23	47.8	14.1	
< 5	124	67.4	6.1		168	65.6	5.7	
Morphology				< .001				.001
Yes	23	29.0	14.1		7	14.3	13.2	
Uncertain	76	59.8	8.3		19	66.3	14.5	
No	81	74.0	7.1		120	75.4	5.8	
PCR (all transcripts), %				.75				.67
≥ 1	7	71.4*	38.2		4	100	0	
0.1 to < 1	13	84.6	19.2		11	81.8	15.6	
< 0.1	35	85.7	8.4		51	82.3	8.7	
CBF, %				.23				.78
≥ 1	7	71.4*	38.2		4	100	0	
0.1 to < 1	9	100	0		10	90	12.7	
< 0.1	20	90	9.0		31	87.1	9.9	
MLL, %				.009				.059
≥ 1	0	0	0		0	0	0	
0.1 to < 1	2	0†	0		1	0†	0	
< 0.1	14	78.6	16.3		17	70.6	17.1	

Abbreviations: CBF, *AML1-ETO* and *CBFβ-MYH11* transcripts; EFS, event-free survival; PCR, polymerase chain reaction; SD, standard deviation.

\*Four-year EFS is shown, because no patient in this group was observed for 5 years.

†Patients died within 1 year.

immunophenotype identical to the one determined at diagnosis. In an additional five MRD-negative samples by PCR, MRD was detected by flow cytometry but < 0.1% (median, 0.06%; range, 0.02% to 0.07%). Surprisingly, only 19 (9.6%) of the 197 samples regarded as MRD positive by PCR were also MRD positive by flow cytometry (median, 0.92%; range, 0.1% to 25.1%); an additional eight samples were positive by flow cytometry < 0.1% (median, 0.04%; range, 0.01% to 0.09%). Thus, 170 (86.3%) of 197 samples with detectable fusion transcripts by PCR did not have detectable leukemic cells by flow cytometry. Even when we restricted the analysis to samples with ≥ 0.1% MRD by PCR (79 of 197), the proportion of those containing AML cells by flow cytometry (including at < 0.1%) remained low: 16 (20.3%) of 79.

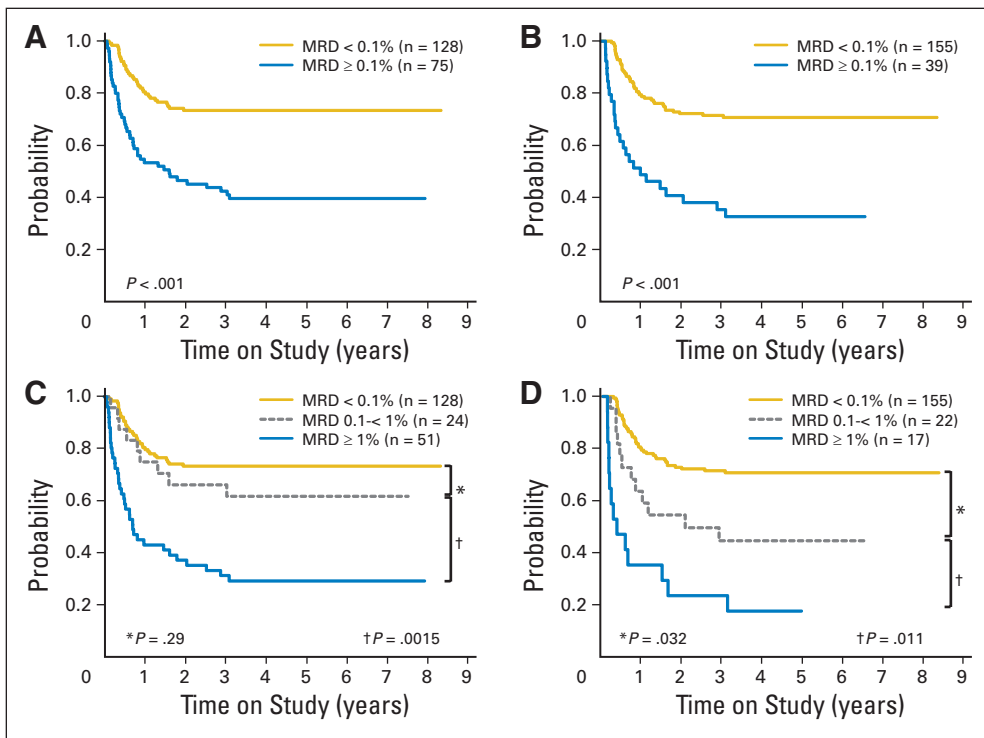
Analyses of *AML1-ETO* or *CBFβ-MYH11* transcripts accounted for most discrepancies between PCR and flow cytometric results. Of the 179 samples that were MRD positive by PCR in this group, only 13 (7.3%) were MRD positive by flow cytometry, and four also had abnormal cells < 0.1% (Figs 3B and 3C); among the 69 that were ≥ 0.1% by PCR, only eight (11.6%) had detectable AML cells by flow cytometry. There was a better correlation in samples positive for the presence of *MLL* fusion transcripts (Fig 3D); six of these 13 samples were MRD positive by flow cytometry, including four of the seven that were ≥ 0.1% by PCR. In analyses limited to samples collected at the end of induction 1, there was agreement between flow cytometry and PCR on *MLL* fusion transcripts (all four positive samples had abnormal cells detected by flow cytometry) but not with PCR on the other transcripts.

### Relation Between Treatment Response by Different Approaches and Outcome

Table 1 shows the results of univariate analyses relating EFS with treatment response after induction 1 or 2 as measured by the three methods (ie, flow cytometry, morphology, and PCR). In line with a previous report,<sup>24</sup> this updated analysis confirmed the prognostic importance of flow cytometric measurements of MRD; EFS rates were significantly worse in patients with MRD ≥ 1% after induction 1 and/or ≥ 0.1% after induction 2 (Table 1; Figs 4A to 4D). A majority of events were leukemia relapses (Data Supplement).

Detection of ≥ 5% myeloblasts in bone marrow smears by morphology was generally associated with EFS ( $P = .016$  after induction 1;  $P = .058$  after induction 2; Table 1). Qualitative assessment based on pathologist impression regarding the presence or absence of residual disease was significantly correlated with EFS ( $P < .001$  after induction 1;  $P = .001$  after induction 2; Table 1). We determined whether morphologic assessment could refine the prognostic value of flow cytometric measurements of MRD. Percentage of myeloblasts by morphology did not affect the relation between MRD by flow cytometry and treatment outcome (Figs 5A and 5B), nor did assessment of the presence of blasts (Data Supplement). By contrast, MRD determined by flow cytometry was a significant predictor of relapse regardless of the morphologic result (Figs 5C and 5D).

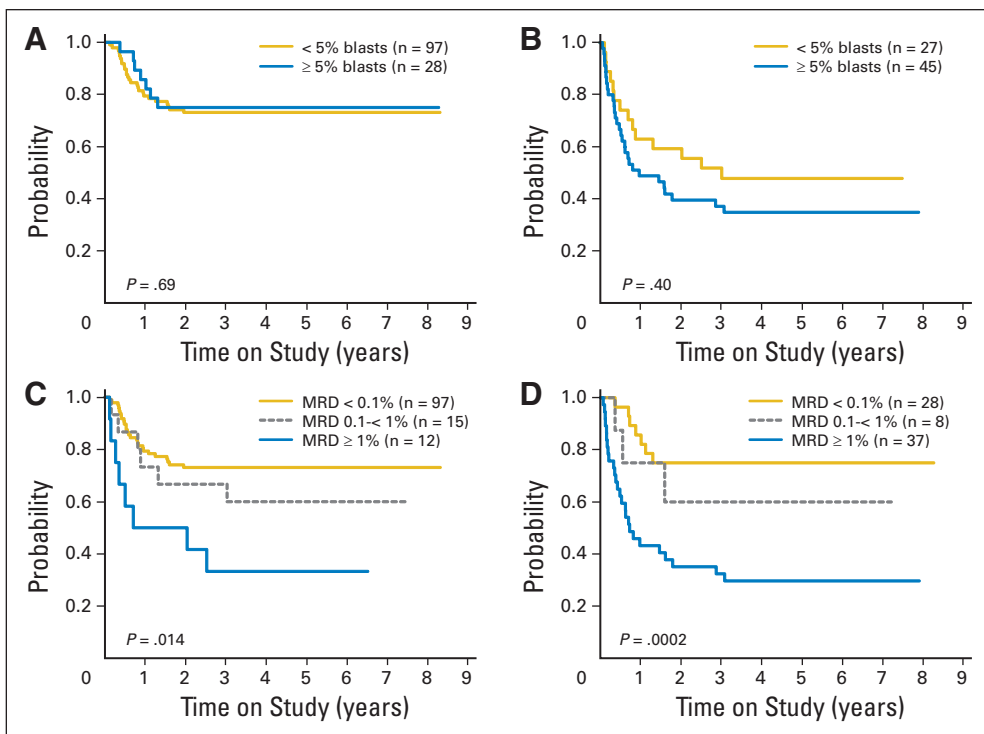
MRD measurements by PCR, when considered collectively using the same MRD cutoff levels used for flow cytometry, were not significantly related to EFS (Table 1). Likewise, a lower cutoff level (0.01%) did not yield significant correlations with outcome ( $P = .63$  after



**Fig 4.** Event-free survival (EFS) for patients with acute myeloid leukemia included in this study according to minimal residual disease (MRD) by flow cytometry. EFS according to presence ( $\geq 0.1\%$ ) or absence ( $< 0.1\%$ ) of MRD (A) after induction 1 and (B) after induction 2. EFS according to levels of MRD (C) after induction 1 and (D) after induction 2.

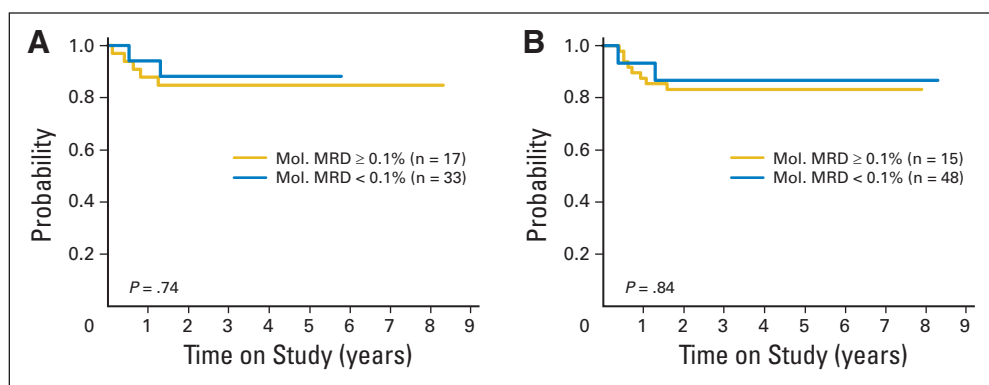
induction 1;  $P = .73$  after induction 2). Moreover, detection of MRD by PCR did not identify a subset of patients with worse outcome among patients for whom MRD negativity was identified through flow cytometry, whether using the 0.1% (Figs 6A and 6B) or 0.01% cutoff (Data Supplement). Only three of the 55 patients with core-

binding factor abnormalities (31 with *AML1-ETO*; 24 with *CBF $\beta$ -MYH11*) in this series relapsed (Data Supplement). One patient with *AML1-ETO* relapsed 1 year after diagnosis; MRD had been negative by both PCR and flow cytometry up to 3 months before relapse. In another patient with the *CBF $\beta$ -MYH11* gene fusion,



**Fig 5.** Relation between event-free survival (EFS) for patients with acute myeloid leukemia included in this study according to flow cytometry and morphology results after induction 1. EFS of patients (A) who were minimal residual disease (MRD) negative ( $< 0.1\%$ ) by flow cytometry according to percentage of blasts by morphology, (B) who were MRD positive ( $\geq 0.1\%$ ) by flow cytometry according to percentage of blasts by morphology, (C) with  $< 5\%$  blasts by morphology according to MRD levels by flow cytometry, and (D) with  $\geq 5\%$  blasts by morphology according to MRD levels by flow cytometry.





**Fig 6.** Event-free survival (EFS) among patients who were minimal residual disease (MRD) negative (< 0.1%) by flow cytometry at the end of (A) induction 1 or (B) induction 2 according to levels of fusion transcripts (*AML1-ETO*, *CBFβ-MYH11*, *MLL-AF9*, *MLL-AF10*, *MLL-ENL*, *MLL-ELL*, or *RBM15-MKL1*) above or below the 0.1% threshold. Analyses using the 0.01% threshold are shown in the Data Supplement. Mol., molecular.

PCR MRD levels rose from 0.005% during follow-up to 1% 10 months after the end of therapy; at this time, MRD also became detectable by flow cytometry (0.24%), and morphologic relapse occurred 2 months later. The third patient with *CBFβ-MYH11* had an isolated CNS relapse before the third consolidation; at the last time point at which MRD was measured in the bone marrow (6 weeks before relapse), it was undetectable by flow cytometry and was 0.1% by PCR. Thirty-five of the 55 patients (all in complete remission at the time of this analysis) had detectable transcripts (estimated MRD levels: 0.0005% to 10%) beyond completion of induction 2, which persisted after completion of therapy in 24 patients. One additional patient with *CBFβ-MYH11* had persistent molecular MRD after initiation of treatment; MRD became detectable by flow cytometry (3.2%) 8 months after the end of therapy, and the patient underwent allogeneic hematopoietic cell transplantation, after which MRD became undetectable by both PCR and flow cytometry.

The relation between *MLL* transcript detection and relapse was different than that observed for *AML1-ETO* and *CBFβ-MYH11*. Of 16 patients studied after induction 1, two had MRD > 0.1%, and both relapsed within 1 year, whereas relapse occurred in three of the 14 patients with MRD lower or undetectable at this time point ( $P = .009$ ). The cutoff level with the highest predictive power for *MLL* fusion transcripts was 0.05% after induction 1 (log-rank  $P = .012$ ) and after induction 2 ( $P = .063$ ). Of note, one *MLL-AF10* patient had detectable MRD by flow cytometry (0.53%) after induction 2 but no detectable *MLL-AF10* transcripts at that time point and relapsed 3 months after the end of therapy. None of the three patients with *RBM15-MKL1* relapsed, despite positive PCR findings in two patients at the end of induction 1 (0.1% and 0.5%) and in one patient at the end induction 2 (0.05%).

We performed a multivariable analysis including measurements of treatment response by flow cytometry and by morphology as well as all presenting features previously shown to be associated with adverse outcome in this cohort: absence of *AML1-ETO* or *CBFβ-MYH11* abnormalities, M7 AML without t(1;22), and *FLT3-ITD*.<sup>24</sup> MRD  $\geq 0.1\%$  by flow cytometry remained a significant predictor of outcome ( $P = .014$  after induction 1;  $P = .013$  after induction 2). Presence of  $\geq 5\%$  blasts by morphology after induction 1 was not a significant predictor ( $P = .39$ ), whereas it was after induction 2 ( $P = .042$ ). Of the other factors, only absence of *AML1-ETO* or *CBFβ-MYH11* abnormalities remained significant ( $P < .01$ ; Data Supplement).

## DISCUSSION

In patients with AML, response to initial treatment is used to determine intensity of subsequent therapy and to select candidates for allogeneic stem-cell transplantation.<sup>24,28-33</sup> Therefore, uncertainties regarding the relative predictive value of different approaches to measure response can affect clinical management, particularly when the results are apparently contradictory. In this study, we compared data obtained by morphologic examination of bone marrow smears, flow cytometric analysis of aberrant immunophenotypes, and PCR amplification of oncogenic fusion transcripts. We found a poor correlation among the three methods overall. A considerable proportion of samples with no clear blasts by morphology or uncertain morphology had leukemic cells by flow cytometry, whereas most samples with residual *AML1-ETO* or *CBFβ-MYH11* transcripts had flow cytometry-negative results. MRD levels by flow cytometry strongly correlated with clinical outcome. Most patients with leukemic blasts identifiable unequivocally by morphology also had high levels of MRD by flow cytometry and had a high relapse hazard. Outcome prediction by PCR MRD was mixed; detection of *MLL* fusion transcripts predicted relapse, whereas *AML1-ETO* and *CBFβ-MYH11* transcripts persisted in many patients who remained in long-term remission.

Our results illustrate the limitations of assessing treatment response by morphology; 17% of the samples had uninterpretable morphology, and 9.5% of those considered to be leukemia free had leukemic cells by flow cytometry, exceeding 1% in several cases. Grouping by percentage of myeloblasts using the 5% cutoff, a strategy used for risk classification in many studies,<sup>34,35</sup> was significantly related to EFS when assessed after induction 1 but did not add useful prognostic information to flow cytometric MRD classification. Conventional cytogenetic analysis may help clarifying the nature of myeloblasts, particularly when these are present in relatively high percentages, and it has been shown to provide prognostic information.<sup>36</sup> However, the sensitivity of this approach (approximately 5%) is limited, as compared with that of flow cytometry.

Previous studies of adult patients with AML have shown that quantitative PCR studies of *AML1-ETO* and *CBFβ-MYH11* can identify patients at a higher risk of relapse, particularly when high transcript levels are detected during consolidation therapy or later.<sup>14-23</sup> However, Lane et al<sup>37</sup> observed that levels of these transcripts after induction and consolidation chemotherapy were not predictive of outcome, but a  $\geq 1 \log^{10}$  rise correlated with inferior leukemia-free

survival, suggesting that MRD monitoring by this approach may be informative only if sequential measurements are performed. The basis for the poor relation between detection of *AML1-ETO* and *CBFβ-MYH11* transcripts, flow cytometric results, and relapse in our study is unclear. Conceivably, low levels of MRD by PCR (undetectable by flow cytometry) could be controlled by subsequent chemotherapy or immune reconstitution and hence fail to predict relapse. Alternatively, the presence of fusion transcripts could result from the persistence of preleukemic cells or from partially differentiated leukemic cells that have lost not only their aberrant immunophenotypic features but also their clonogenic potential.

The prognostic value of MRD monitoring targeting *MLL*-rearranged transcripts in AML has not been extensively studied. Scholl et al<sup>38</sup> studied 19 patients and observed that the 11 with consistently negative PCR findings had lower relapse rates than the remaining eight. Among the 16 patients with *MLL* transcripts whom we studied at induction 1, the two who had MRD > 0.1% relapsed within 1 year. By contrast, 13 of the 18 patients with *MLL*-AML with persistently negative PCR MRD after induction 2 are still in remission at the time of this report. Although there was a much better correlation between flow cytometric results and detection of *MLL* transcripts as compared with *AML1-ETO* and *CBFβ-MYH11* transcripts, this was not absolute; six of 13 *MLL*-positive samples were MRD positive by flow cytometry, whereas two additional samples had cells just below the 0.1% threshold; conversely, three of the 119 PCR-negative samples were positive by flow cytometry.

There are other potential targets for molecular studies of MRD in AML that were not used in this study. The Wilms tumor gene (*WT1*) is highly expressed in most acute leukemias, and its detection in bone marrow has been associated with relapse,<sup>39-42</sup> but the background of normal bone marrow cells with *WT1* expression limits the applicability of this assay.<sup>43</sup> Another potential target for molecular studies is *FLT3-ITD*, which, in line with a previous study,<sup>44</sup> was present in 13% of patients in our cohort. However, it has been reported that this marker may not be stable during the course of disease,<sup>45,46</sup> reducing its

value in MRD monitoring. Finally, nucleophosmin mutations are a promising target for MRD monitoring,<sup>47</sup> but less than 10% of children with AML have this abnormality.<sup>48</sup>

In sum, our study shows that measurements of treatment response by flow cytometry are widely applicable and provide strong prognostic information; in the subset of patients with *MLL* gene abnormalities, monitoring of *MLL* fusion transcripts is also clinically useful. By contrast, the value of morphologic monitoring is limited, and PCR results of *AML1-ETO* and *CBFβ-MYH11* are difficult to interpret. We would argue that the use of such tests to select treatment strategies should be undertaken with caution or could be abandoned altogether, particularly if a reliable flow cytometric assay is available.

#### AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

#### AUTHOR CONTRIBUTIONS

**Conception and design:** Hiroto Inaba, Elaine Coustan-Smith, Stanley B. Pounds, Jeffrey E. Rubnitz, Dario Campana

**Financial support:** Hiroto Inaba, James R. Downing, Ching-Hon Pui, Dario Campana

**Administrative support:** Raul C. Ribeiro, James R. Downing, Ching-Hon Pui

**Provision of study materials or patients:** Hiroto Inaba, Elaine Coustan-Smith, Sheila A. Shurtleff, Susana C. Raimondi, Mihaela Onciu, Raul C. Ribeiro, Gary V. Dahl, W. Paul Bowman, Jeffrey W. Taub, Barbara Degar, Wing Leung, James R. Downing, Jeffrey E. Rubnitz

**Collection and assembly of data:** Hiroto Inaba, Elaine Coustan-Smith, Sheila A. Shurtleff, Kathleen Y. Wang, Dario Campana

**Data analysis and interpretation:** Hiroto Inaba, Elaine Coustan-Smith, Xueyuan Cao, Stanley B. Pounds, Dario Campana

**Manuscript writing:** All authors

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